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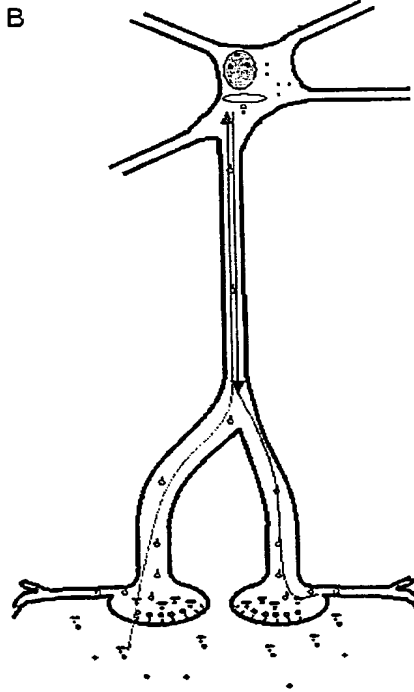
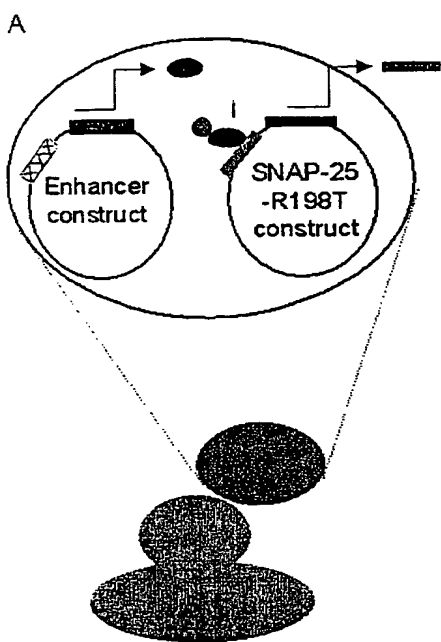
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LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,

[Continued on next page]

(54) Title: ISOFORMS OF SNARE MOLECULES AND THE USES THEREOF IN MODULATION OF CELLULAR EXOCY-  
TOSIS



(57) Abstract: A method of treating a patient suffering from poisoning by clostridial toxin wherein a SNARE (soluble (N-ethylmaleimide-sensitive fusion protein)-attachment protein receptor) that is resistant to proteolysis by the said clostridial toxin (toxin-resistant SNARE) and/or is capable of inhibiting the clostridial toxin is supplied to a cell of the patient. The SNARE that is resistant to proteolysis may be, synaptosomal-associated polypeptide of 25 kDa (SNAP-25). The SNAP-25 is preferably resistant to proteolysis by BoNT/A, BoNT/E and BoNT/C. A method of treating a patient in need of inhibition of SNARE-dependent exocytosis from a cell capable of performing SNARE-dependent exocytosis wherein a derivative (inhibitory SNARE) that is capable of inhibiting SNARE-dependent exocytosis is supplied to the said cell of the patient. The inhibitory

SNARE may be a fragment of SNAP-25 that is derivable by cleavage of SNAP-25 by botulinum toxin A (BoNT/A). The cell may be, for example, a nerve cell, adreno-chromaffin cell or insulin-secreting cell. The SNARE may be supplied to the cell by expressing recombinant polynucleotide construct. The SNARE or construct may be targeted to a nerve cell, by means of an inactive clostridial neurotoxin. The SNARE may be expressed under the target cell-specific promoter.

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NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

68 00/03196

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/62 C07K14/705 A61K38/17 A61K38/57 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GONELLE-GISPERT C., HALBAN, P. A., NIEMANN H., PALMER M., CATSICAS S., SADOUL K.: "SNAP-25a and -25b isoforms are both expressed in insulin-secreting cells and can function in insulin secretion." BIOCHEMISTRY JOURNAL, vol. 339, 1 April 1999 (1999-04-01), pages 159-165, XP002159203 see page 163 "Production of BoNT/E-resistant SNAP-25 isoforms"; page 159 "Abstract"; p. 162 "Functional role of SNAP-25 isoforms in insulin secretion"; p.160 "Mutagenesis of SNAP-25 isoforms"	2-4,6, 12-15, 25,30, 32,34, 36-39
Y	see passages cited above	1,5, 8-11,16, 26-28, 33,35, 40-45
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "1" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the international search

7 February 2001

Date of mailing of the international search report

23.04.01

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# INTERNATIONAL SEARCH REPORT

International Application No.

GB 00/03196

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SADOUL K., BERGER A., NIEMANN H., WELLER U., ROCHE P.A., KLIP A., TRIMBLE W.S., REGAZZI R., CATSICAS S., HALBAN P.A.: "SNAP-23 is not cleaved by botulinum neurotoxin E and can replace SNAP-25 in the process of insulin secretion" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 52, 26 December 1997 (1997-12-26), pages 33023-33027, XP002159204 see page 33026 "Discussion"; p. 33024 "SNAP.23 is resistant to cleavage by BoNT/E"	2-4,6, 12-15, 25,32,36
Y	see passages cited above	1,5,10, 11,16, 26-29, 31,33, 35,44,45
Y	--- BRUNSD D., ENGERS S., YANG C., OSSIG R., JEROMIN A., JAHN R.: "Inhibition of transmitter release correlated with the proteolytic activity of tetanus toxin and botulinum toxin A in individual cultured synapsys of Hirudo medicinalis" JOURNAL OF NEUROSCIENCE, vol. 17, no. 6, 15 March 1997 (1997-03-15), pages 1898-1910, XP002159205 see page 1898 "Abstract" and "Introduction"; p. 1902 "Leech SNAP-25 is resistant to cleavage by BoNT/A"; Figure 2B; p.1908 right-hand-side column	1,5, 8-11,16, 29,31, 33,35, 44,45
Y	--- WO 95 32738 A (ALERGEN) 7 December 1995 (1995-12-07) cited in the application see page 2 "Summery of the invention" -----	26-28, 40-43

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 00/03196

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 1-5, 8-16, 36 and 47 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

1-5(partly), 8-16(partly), 25-39(partly), 42-45(partly) and 47(partly)

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-5(partly), 8-16(partly), 25-39(partly),  
42-45(partly) and 47(partly)

are directed towards forms of SNARE molecules resistant to cleavage by clostridial toxins, their uses and polynucleotides coding therefor.

2. Claims: 1-18(partly), 25-39(partly) and 42-47(partly)

are directed towards forms of SNARE molecules capable of blocking the proteolytic activity of clostridial toxins, their uses and polynucleotides coding therefor

3. Claims: 25-28(partly), 30-39(partly),  
42-44(partly) and 46-47(partly)

are directed towards forms of SNARE molecules capable of inhibiting SNARE-mediated exocytosis, their uses and polynucleotides coding therefor.

4. Claims: 26-28(partly), 30(partly), 33-35(partly),  
37-39(partly), 40, 41,  
42-44(partly) and 47(partly)

are directed towards a gene therapy delivery system based on proteolytically inactive form of clostridial toxin.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

GB 00/03196

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9532738	A	07-12-1995	AU 695623 B	20-08-1998
			AU 2622295 A	21-12-1995
			CA 2191754 A	07-12-1995
			DE 69511860 D	07-10-1999
			DE 69511860 T	10-02-2000
			DK 760681 T	27-03-2000
			EP 0760681 A	12-03-1997
			ES 2138740 T	16-01-2000
			JP 10500988 T	27-01-1998
			US 6203794 B	20-03-2001